PARTICLE GUN TRANSIENT EXPRESSION OF SEXUAL EMBRYOS OF COMMON BEAN

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The fact that more efficient methods in plant breeding are needed has been discussed for several years. The use of biotechnology in plant breeding may prove to be a more cost effective means to alter the genomes of economically important crops than through conventional methods. Common bean (Phaseolus vulgaris L.) is one of the most recalcitrant crops to genetically alter through biotechnology. Regeneration from somatic cells has not been successful in common bean.

Particle acceleration is one of the promising new gene transfer techniques(11). In this system genetic alteration of plants is mediated through the bombardment of plant cells with microsized tungsten particles coated with DNA. The DNA coated particles are accelerated to velocities sufficient for the nonlethal penetration of cell walls and membranes. Several laboratories have used particle acceleration and have produced stable transgenic tissues and plants. Most often transient expression of reporter genes, eg, GUS, is accomplished. Stable or transient transformation has been demonstrated in tobacco(5), rice(2), soybean(3) , barley(8), wheat(4), maize(7), and papaya(6) using plant explant or suspension cultures.

Since the methodologies used today have been unsuccessful in effecting regeneration of common bean plants from somatic explants, particle acceleration may overcome this roadblock. The objective of this research was to ascertain whether or not particle acceleration can be used to transform common bean.

MATERIALS AND METHODS.

Axes with the leaf meristems removed from sexual embryos of 'ICA pijao' were exposed to particle acceleration. The embryos were oriented with stem primordia upward. The axes of embryos were removed from the seed after two or three days of germination in Petri dishes. Batches of 35 freshly isolated embryo axes were placed in a compact cluster in 15 x 60 mm Petri plates on a semi-solid nutrient medium. All procedures were carried out aseptically.

A plasmid was used as the vehicle to carry the DNA encoding for the ß-glucuronidase, and kanamycin resistance genes, and a gene for resistant to the herbicide, Basta. The plasmid we used was pGV1040 and constructed in Montagu's laboratory(1).

Tungsten particles coated with DNA were prepared essentially according to the protocol described by Klein et al, 1988(9). Plasmid (PGV1040) DNA was absorbed onto 2 mg of tungsten particles by adding 5 µl of DNA (2 µl/µg or more) to 25 µl suspension of the tungsten particles in the presence of $CaCl_2$ (25 µl of a 2.5 M solution) and spermidine free base; 10 µl of a 0.1M

solution in microfuge tubes.

Three distances from the stopping plate to target tissue were tested: 5, 10 and 18 cms. We also tested the efficacy of single, double and triple bombardments on the same target tissue.

The substrate we used to obtain GUS expression was prepared according to Kosugi et al, 1990 (10). Explants were placed in microfuge tubes containing 50 mM NaH_2PO_4, 10 µM EDTA, 0.3 M Manitol (pH 7.0), 1 mM X-Glu (5-bromo-4-chloro-3-indolyl ß-D glucuronic acid) in 20% methanol.

RESULTS AND DISCUSSION

The distance of the recipient embryo clusters to the stopping plate affected the expression of GUS activity. The highest level of GUS activity (in terms of color intensity) in cultured embryos axes was observed when the distance between the stopping plate and the target explant was 10 cm. However, the highest number of impacts was observed at 5.0 cm. At 18 cm, explants did not show GUS activity. GUS expression was detected as early as 5 days after bombardment. Two bombardments were required for obtaining a satisfactory GUS expression.

Although 5 cm was the distance where more expression was obtained in terms of impacts, the 10 cm distance was used for the experiment to ascertain the most efficient number of bombardments per plate.

One bombardment gave a low level of gus expression. We found that two bombardments of the target tissue the most acceptable in terms of level of GUS expression and minimal damage. This finding is in disagreement with Kartha et al, 1989 (8) who found that double bombardment had deleterious effects on cells of immature embryos. Klein et al, 1988 (9) found that three bombardments gave a higher GUS expression than one or two bombardments. However, in our case, while three bombardments of the target tissue gave the same level of GUS expression as two bombardments, the amount of cellular damage was unacceptable.

Particle acceleration appears promising as a means to direct foreign DNA into the genome of common beans and recover transgenic plants. However, before particle acceleration becomes a useable technology, work is needed to improve the method's efficiency in terms of concentration of bombardments.

REFERENCES

1. Calderon, A. 1988. Mc Thesis, Urige Universiteit. Brussile. Brussels, Belgium.
3. Christou, P., et al. 1991. Biotechnology, 9: 957-962.
4. Christou, P., et al. 1988. Plant Physiol, 87: 671-674.
5. Daniel, H., et al. 1991. Plant Cell Reports. 9:615-619
6. Dwight, T., et al. 1990. Plant Mol. Biol. 14: 261-268.
7. Fitch, M.M.M., et al. 1990. Plant Cell Rep. 9: 189-194.
8. Gordon-Kamm, W.J., et al. 1991. In Vitro Cell. Dev. 27:21-27.
9. Kartha, K.K., et al. 1989. Plant Cell Reports. 8:429-432.
10. Klein, T.M., et al 1988. Proc. Natl. Acad. Sci. USA. 85: 8502-8505.
11. Kosugi, S., et al. 1990. Plant Science, 70: 133-240.
12. Sanford, J.C. 1988. Trends Biotechnol. 6: 299-302.